369

MicroRNA-132 supports wound healing by enhancing inflammatory-proliferative phase transition

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To understand the role(s) of microRNAs in skin wound healing, we characterized the dynamic change of the miRNome in human skin wounds. One of the top-up-regulated miRNAs in the inflammatory phase of wound repair, miR-132, was found to be predominantly expressed in epidermal keratinocytes and its level peaked in the subsequent proliferative phase. We show that both TGF-β1 and TGF-β2 induce miR-132 expression in vitro and in vivo. Transcriptome analysis revealed that miR-132 regulated a large number of immune response- and cell cycle-related genes in keratinocytes. Through overexpression or inhibition of miR-132, we found that miR-132 decreased the production of chemokines by keratinocytes and their capability to attract leukocytes by suppressingthe NF-κB pathway. On the other hand,miR-132 promoted keratinocyte growth by increasing the activity of the STAT3 and ERK pathways. Silencing of miR-132's target, heparin-binding EGF-like growth factor (HB-EGF),phenocopies the effects of overexpressing miR-132 in keratinocytes. In vivo, miR-132 KO mice show thinner epidermis with less proliferating cells in comparison with their WT littermates. Upon injury, we observed higher level of chemokine/cytokine expression, more neutrophils infiltration and decreased proliferation of epidermal keratinocytes in the wounds of the KO mice compared with the WT mice. Moreover, we injected miR-132 inhibitors into the wound edges of WT mice, which also showed impaired wound healing, which confirmed the phenotype in miR-132 KO mice. We also validated these mouse data in human skin wounds using a human ex vivo wound model. Together, We conclude that miR-132 is a critical regulator of skin wound healing through facilitating the transition from the inflammatory to the proliferative phase.

370

Nrf2 activation promotes cutaneous wound healing by keratinocyte protection and stem cell activation

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The transcription factor Nrf2 is a key regulator of the cellular stress response through the regulation of antioxidant enzymes, cytoprotective proteins and various transporters, including multidrug resistance proteins. Accordingly, pharmacological activation of Nrf2 is a promising strategy for skin protection and cancer prevention in this tissue. Surprisingly however, little is known about the effect of Nrf2 activation on wound repair. Therefore, we analyzed the cutaneous wound healing process in transgenic mice expressing a constitutively active (ca) version of Nrf2 in keratinocytes. We observed enhanced wound closure in these mice due to an increased length and area of the wound epithelium. The proliferation of keratinocytes was unchanged, but Nrf2 targets involved in ROS detoxification were significantly upregulated. In addition, we observed an increased number of Lrig1^{+}junctional zone stem cells in hair follicles of unwounded mice, which correlates with the thickened infundibulum and hyperplasia of sebaceous glands also observed. Since infundibular stem cells migrate into the wound, we speculate that Nrf2 enhances wound closure by an expansion of the Lrig1+ stem cell pool and their subsequent migration into the wound. We suggest that the underlying mechanism involved is the upregulation of the EGF family member epigen, which we recently identified as a direct Nrf2 target and which has been shown to cause an expansion of the Lrig1+ stem cell population. Taken together, our data demonstrate that Nrf2 activation enhances wound closure by protection of migrating keratinocytes inside the wound, as well as the expansion of the Lrig1+ follicular stem cell population.

371

Atypical Kinase C balances stem cell renewal and differentiation through the tumor suppressor Lethal Giant Larvae (Lgl)

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Self-renewing epithelia need to balance proliferation and differentiation to avoid disease states as cancer or premature ageing. During the last decades the importance of coupling mitotic spindle orientation to cell fate determinants to control these decisions emerged. We previously identified atypical kinase Cλ (aPKCλ) as a key regulator of epidermal stem cell fate decisions, likely by balancing self-renewal promoting symmetric and differentiation promoting asymmetric divisions. To further investigate if and how aPKCλ links spindle orientation to cell fate we generated a transgenic mouse model allowing epidermis-specific expression of membrane-targeted aPKCλ (aPKCλ^{CAAX}). In *Drosophila* neuroblasts, aPKC^{CAAX} promotes symmetric division, stem cell renewal and tissue overgrowth. Interestingly, similar to epidermal loss of aPKCλ, an increase in asymmetric spindle orientation was observed in the epidermis of aPKCλ^{CAAX} mice. However, aPKCλ^{CAAX} expression increased stem cell numbers and a prolonged hair follicle resting phase, phenotypes opposite from those of aPKCλ$^{epi-/-}$ mice. Thus, either spindle orientation does not determine epidermal cell fate or fate determinants are differently coupled to spindle orientation upon loss or membrane targeting of aPKCλ. To identify how aPKC controls cell fate we performed (phospho)-SILAC and immunoprecipitation analysis together with proteomics and identified the tumor suppressor lethal giant larvae (Lgl), previously implicated in cell fate decisions in *Drosophila*, as a major aPKCλ binding partner. Interestingly, Lgl protein stability and localization, also in mitotic anaphase cells, was altered in opposite directions in aPKCλ$^{epi-/-}$ versus aPKCλ^{CAAX} mice. Initial rescue experiments indicate that aPKCλ might regulate epidermal cell fate through Lgl. Together, the data show that aPKC is a crucial regulator of epidermal stem cell fate potentially through Lgl and that spindle positioning cannot explain epidermal cell fate decisions.

372

Role of the polarity protein Par3 in mammalian skin homeostasis and regeneration

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Tissue homeostasis and regeneration require concerted cell polarization and tissue architecture. The evolutionarily conserved and ubiquitously expressed components of the Par complex namely, *Par*titioning-defective 3 (Par3), atypical protein kinase C (aPKC) and Par6 play a major role in the regulation of various cell polarization processes. Recently, impaired functions of Par3 and aPK-Clambda have been shown to differentially affect spindle orientation in mammals, suggesting that Par3 and aPKC isoforms may not only have common but also distinct roles in certain tissues. To assess a potential function of Par3 in skin homeostasis and regeneration, we generated mice with epidermal Par3 deletion (Par3 eKO). Characterization of Par3 eKO mice at different developmental and adult stages revealed increased transepidermal water loss, delayed tight junction formation, and increased epidermal differentiation and thickness. Par3 deletion also resulted in enlarged, multilobular sebaceous glands and progressive loss of hair follicle bulge stem cells suggestive of altered cell fate decisions and premature ageing. These results indicate that Par3 regulates the overall cell fate by controlling the balance of stem cell maintenance vs. differentiation in the epidermis and hair follicle. Moreover, loss of Par3 results in impaired keratinocyte migration *in vitro* and delayed wound closure *in vivo* with abnormal deposition of ECM components such as Collagen IV. Par3 thus appears to be important for epidermal regeneration processes in the skin. Together, our data suggest that the conserved polarity protein Par3 controls both the maintenance and regeneration of an important self-renewing and barrier-forming stratified epithelium in mammals. Note: This abstract was presented at the International Meeting of the German Society of Cell Biology (Deutsche Gesellschaft für Zellbiologie (DGZ), 2015.

373

Ceramide synthase 4 affects hair follicle cycling and stem cell maintenance

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Lipids are a vital part of the epidermis and ceramides are quantitatively and for structural reasons most important for the epidermal permeability barrier. Ceramides are in addition key components of mammalian cell membranes and important players in signaling pathways. Ceramide production depends on ceramide synthases (CerS). However, little is known on the role of CerS and ceramides in the formation and maintenance of epidermal appendages and whether stem cell populations that control epidermal regeneration depend on specific ceramide species. Our investigation shows that ceramide synthase 4 (CerS4) is highly expressed in adult murine epidermis where it is localized in the interfollicular epidermis and specific compartments of the hair follicle. Inactivation of CerS4 induced precocious activation of hair follicle bulge stem cells. This was manifested in a continuous anagen-like growth state of hair follicles after the second catagen and a loss of label retaining cells. This ultimately led to an almost complete depletion of bulge stem cells in one-year old mice. At the second catagen to telogen transition a reduction in BMP target gene expression was identified, indicating a decrease in BMP signaling in CerS4$^{-/-}$ epidermis. This may explain the inability of hair follicle stem cells to properly enter telogen. Further the reduction in BMP activity likely promotes subsequent enhanced Wnt target gene expression. Our data reveal an essential role of CerS4-directed epidermal ceramide composition in the control of hair follicle stem and progenitor cell activation and dynamics. Thus our data suggest a novel means of hair follicle stem cell activation, which is of relevance for understanding the regulation of adult stem cell populations.

374

b-catenin signalling influences melanocyte and Schwann cell specification from the bipotent progenitors following the ventral migration pathway

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Recent works suggest a dual neural crest origin of melanocyte directly from neural crest cells (NCC) via the dorsolateral pathway (first wave), or indirectly from NCC-derived Schwann cell precursors (SCPs) attached to peripheral nerves in the ventral pathway (second wave). In this study we aim to better characterize the melanoblasts originating from the ventral pathway and define the influence of the Wnt/b-catenin signaling on the specification between melanoblasts and Schwann cells from the SCP precursors. We first generated transgenic mice expressing a stabilized form of b-catenin after specification of the melanoblast from the dorsolateral pathway. Interestingly these mice (Tyr::Cre/°; bcatex3$^{flox/+}$ = bcat-Dex3) did not present any pigmentation phenotype at the level of the coat, ear or tail but displayed high levels of pigmentation associated with ectopic melanocytes in the palms and soles. By using a spatio-temporal controlled mouse system (Tyr::CreERt2; bcatex3$^{flox/+}$) we could show that ectopic palm and sole melanoblasts were present once b-catenin was stabilized at the time of second wave melanoblasts specification and after the specification of first wave melanoblasts. Using 3D imaging technology of wild-type paws, until E13.5 cells are coexpressing MITF (melanoblast marker) and GFAP (Schwann cell marker). At later stages, cells are either MITF-positive or GFAP-positive. This suggests that prior E13.5, these cells are bipotent prior being specified as melanoblasts or Schwann cells. Interestingly from E14.5, bcat-Dex3 embryos present an increased number of MITF positive cells and a reduced number of GFAP positive cells. Altogether, these results show that an induction of the Wnt/b-catenin pathway of the SCP favour the melanoblast specification.

375

Suppression of Neutrophil-Mediated Tissue Damage – A Novel Skill of Mesenchymal Stem Cells

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Mesenchymal stem cells (MSCs) are crucial for tissue homeostasis and regeneration. Though of prime interest, their potentially protective role on neutrophil-induced tissue damage with high morbidity and mortality is largely unexplored, which precludes rationale therapeutic interventions. Therefore, we set out to investigate the effects of MSCs on neutrophil activation. MSCs significantly reduced the oxidative burst with unrestrained release of reactive oxygen species (ROS) and active neutrophilic enzymes involved in tissue damage including myeloperoxidase, elastase and gelatinase from cocultured activated neutrophils. Interestingly, as shown by confocal microscopy, MSCs engulfed neutrophils in an ICAM-1/beta2 integrin dependent manner, thus preventing the spillage of tissue damaging proteolytic enzymes and ROS. In this regard also the formation of neutrophil extracellular traps was substantially inhibited by MSCs. The suppressive effects of MSCs on activated neutrophils were further confirmed *in vivo*. Intradermally injected MSCs resulted in a 40% reduction of ROS release in a murine model of PMA-induced neutrophilic skin inflammation as detected by *in vivo* imaging with a ROS-sensitive chemiluminescent probe. Similarly, MSC-injection reduced haemorrhage and vessel destruction in a mouse model of IC-mediated neutrophil-dependent vasculitis. MSCs adaptively built up an antioxidant shield via enhanced release of soluble superoxide dismutase SOD3, thus effectively interrupted the vicious cycle of ROS-induced proteolytic and oxidative tissue damage caused by overactivated neutrophils. SOD3 silenced MSCs substantially lost their tissue protective capacity. Thus, MSCs hold substantial promise to counteract unrestrained tissue damage in conditions with overactivated neutrophils.

376

Dominant negative mutation of Sox18 inhibits normal dermal papilla development during embryogenesis and regeneration

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Hair follicle development requires signalling from a specialised region of underlying mesenchyme known as the dermal papilla (DP). The molecular mechanisms by which the DP functions however remains unknown, in particular how it regulates the development of complex tissue patterning in the hair. Using a Sox18-GFP expressing murine model we identify that Sox18 is expressed in the DP of all hair types, in a temporally controlled manner. Further, using the dominant negative $Sox18^{Op/+}$ mouse model our study shows that Sox18 is required in all hair types for normal development. Interestingly, the phenotype of hairs post Sox18 mutation is hair type specific; Guard hairs have a mild growth defect while awl/auchene/zig-zag hair development is completely inhibited. These data support that Sox18 is required for all hair types, though with differential requirements in each. We have also identified a number of potential Sox18 targets via microarray that we will continue to investigate in regards to their role in regulation of hair growth by the underlying DP. These data identify that sox18/soxF factors regulate dermal papilla differentiation and by this mechanism are crucial for hair follicle development.

377

MFG-E8 promotes mesenchymal stem cells-induced angiogenesis

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We previously reported that pericytes are major sources of the secreted glycoprotein and integrin-ligand MFG-E8 in B16 melanoma tumors, and that MFG-E8 promotes angiogenesis via enhanced PDGF receptor β signaling. Recently, there is increasing evidence that pericytes and mesenchymal stem cells (MSC) are similar cells that localize around vasculature and are involved in angiogenesis and tissue repair, suggesting that MFG-E8 might regulate the function of MSC. Our objective was to elucidate the role of MFG-E8 in the regulation of MSC-induced angiogenesis. We first found that mice bone marrow-derived MSC expressed large amounts of MFG-E8. To assess the role of MFG-E8 in MSC-enhanced tumor growth and angiogenesis, B16 melanoma cells and MFG-E8 WT/KO mice-derived MSC were co-implanted subcutaneously into mice. Tumor sizes and numbers of $CD31^+$ vessels and $NG2^+$ pericytes in tumors initiated with melanoma cells and MFG-E8 KO MSC were smaller than those initiated with melanoma cells and MFG-E8 WT MSC. We identified that $MFG\text{-}E8^+$ MSC were localized around blood vessels in melanoma tumors. In wound healing mice model, wound healing and vascular amounts in wound area treated with subcutaneous injection of MFG-E8 KO MSC were also inhibited compared with those treated with MFG-E8 WT MSC. The number of M2 macrophages in melanoma tumor and wound area treated with MFG-E8 KO MSC was smaller than those treated with MFG-E8 WT MSC. In *in vitro* assay, levels of VEGF and ET-1 mRNA in MFG-E8 KO MSC were lower than those in WT MSC. MFG-E8 WT MSC-conditioned medium induced macrophage polarization into M2 type compared to KO MSC-conditioned medium. These results suggest that MFG-E8 might increase the expression of VEGF and ET-1 in MSC as well as the polarization of M2 macrophages, resulting in enhancement of angiogenesis in melanoma and wound. In immunofluorescence studies in human melanomas and wound area, MFG-E8 staining was mainly observed around blood vessels, especially in pericytes, suggesting that MFG-E8 may regulate angiogenesis in human melanoma and wound via pericytes and/or MSC.

378

Role of cortical actin disorganization in keratinocyte proliferation in psoriasis

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Psoriasis is a chronic inflammatory skin disorder characterized by keratinocyte hyper-proliferation. Although hyper-activation of Th17 and Th22-mediated immunities has been considered as a major cause of psoriasis, the precise mechanism of uncontrolled keratinocyte proliferation remains unclear. Recently, actin filament dynamics have been reported to exert an important role in controlling the balance between differentiation and proliferation of keratinocyte. In this study, we found that dense cortical actin fibers were dispersed throughout the cytoplasm of keratinocytes in the basal layer of lesional skin in psoriasis patients. In addition, imiquimod-induced psoriasis-like mouse skin also exhibited a similar change. To understand functional consequences of cortical actin disorganization, we focused on primary cilia, whose biogenesis is intimately linked to actin cytoskeletal remodeling. Primary cilia are cellular organelles sensing diverse extracellular signals. Interestingly, the biogenesis of primary cilia was increased in keratinocytes of psoriatic skin. We further found that ciliogenesis could be induced by either psoriasis-related cytokines or actin dynamics-modulating drugs, Latruculin A, Blebbistatin, Y27632, and a LIM kinase inhibitor. The induction of ciliogenesis was accompanied by an increase in BrdU-positive cells. This finding is consistent with previous reports which showed that inhibitors of Rho-ROCK pathway and actin polymerization induce keratinocyte proliferation. To investigate the role of primary cilia in the pathogenesis of psoriasis, we developed tamoxifen-inducible conditional knockout mouse for IFT20, a crucial gene in ciliogenesis. Remarkably, in tamoxifen treated mice the development of psoriasis-like lesion was attenuated after imiquimod treatment. Our results suggest that disorganized cortical actin in psoriasis lesion promotes aberrant formation of primary cilia, which play a role in the regulation of keratinocyte proliferation.

379

Transient Receptor Potential Vanilloid-4 Inhibits Human Hair Growth

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Transient receptor potential (TRP) ion channel family members are widely expressed in the human skin and are involved in the regulation of numerous cutaneous functions. We have previously shown that certain members of the vanilloid TRP subfamily (TRPV1 and TRPV3) are functionally active on human hair follicles (HF) and induce premature catagen transition. In the current study, we investigated the expression and functional role of another TRP, TRPV4, in human HFs and HF-derived outer root sheath keratinocytes (ORSK) which are thought to play an important role in regulating HF cycling. Gene expression was investigated by RT-qPCR and immunofluorescence. Proliferation, viability, and cell death were assessed by CyQUANT- and MTT-assays and $DiIC_1(5)$-SYTOX Green labeling, respectively. Ca^{2+}-homeostasis was monitored by Fluo-4 AM-based Ca^{2+}-imaging. First, we found that TRPV4 is expressed in human HFs both at the mRNA and protein levels. Moreover, we also showed that the synthetic TRPV4 agonist GSK1016790A increased $[Ca^{2+}]_{IC}$ in a dose-dependent manner, which could be abrogated by the co-application of the selective TRPV4 antagonist HC067047. Interestingly, however, GSK1016790A treatment did not alter the proliferation of the ORSKs, but, at higher concentrations, it decreased the viability by initiating both necrotic and apoptotic processes. Importantly, activation of TRPV4 by GSK1016790A significantly inhibited hair shaft elongation of intact human HFs in a dose-dependent manner, which could partially be reversed by the co-administration of HC067047. Collectively, our findings revealed that TRPV4 is functionally expressed on the human HFs, its activation leads to induction of apoptosis/necrosis of ORSKs, and suppresses hair growth. These results should therefore encourage one to systematically explore putative therapeutic potential of TRPV4-modulators in the management of diseases characterized by pathologically altered hair growth.

380

Lgr6+ stem cells and their progeny in the skin and skin tumours after UV exposure

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Recently, Lgr6+ epidermal stem cells were discovered in mouse skin. These cells reside in the isthmus of the hair follicle and in the interfollicular epidermis (IFE). In this study we investigated responses of Lgr6+ stem cells to UV exposure, specifically whether their progeny a) drives UV-induced hyperplasia, b) repopulates the basal layer of the IFE after ablation by a UV overdose, and c) populates UV induced tumours. We used transgenic mice in which Lgr6 (EGFP+) stem cells could be detected, and their progeny could be traced by tamoxifen-induced irreversible activation of a LacZ reporter gene (activation before UV exposure, or during tumour growth). After induction of hyperplasia and after ablation of the epidermal basal layer, the progeny of Lgr6+ stem cells was found in the same locations as in homeostasis. However, they became less frequent in the IFE during UV induced hyperplasia, as did the Lgr6+ cells themselves. In the UV induced tumours we did not find any Lgr6+ stem cells and their progeny was rarely and sparsely present. Using qPCR on frozen skin tumours we did, however, find low Lgr6 expression that apparently escaped detection by the reporter construct. Lgr6+ stem cells appear to be prominently targeted by UV irradiation since they are present in the IFE. Their numbers decreased after chronic UV exposure and we did not find Lgr6 stem cells or their progeny in tumours. Therefore, these stem cells do not appear to be the drivers of UV-induced skin tumours. Nevertheless, we did detect Lgr6 mRNA from EGFP- cells at low levels and/or from alternative transcripts in a subgroup of tumour cells.

381

Keeping in touch with autophagy – a mouse model with Atg7-deficient Merkel cells

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Merkel cells play essential roles in the soft touch sensation of the skin. Recent studies have revealed that Merkel cells and epidermal keratinocytes develop from a common precursor, but many aspects of the biology of Merkel cells have remained uncharacterized. Here, we report the use of the $Atg7^{f/f}$ K14-Cre mouse model for the comparative investigation of autophagy (lysosomal degradation of cellular components) in Merkel cells and keratinocytes. The essential autophagy gene *Atg7* was deleted by the Cre recombinase under the control of K14 promoter. Transgenic expression of the autophagy reporter GFP-LC3 in $Atg7^{f/f}$ K14-Cre and control mice demonstrated the Atg7-dependent presence of autophagosomes both in keratinocytes and Merkel cells *in vivo*. Immunofluorescence double-labeling showed that the deletion of Atg7 leads to the massive accumulation of the autophagy adaptor and substrate p62/sequestosome 1 in K8-positive Merkel cells of the whiskers. By contrast, keratinocytes of the hair follicle and the interfollicular epidermis showed only a weak, if any, accumulation of p62 under these conditions. Taken together, these results suggest that autophagy is active in normal Merkel cells and that the suppression of autophagy has a stronger effect on the homeostasis of Merkel cells than on keratinocytes.

382

Development of myelinated and non-myelinated sensory nerve fibers in reinnervated human skin promotes maturation of mast cells from resident progenitor cells

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Skin mast cells (MC) are in close contact with nerve fibers (NF), which induce MC maturation in murine skin. However, it is unknown whether this also occurs in human skin. To clarify this, we re-innervated healthy adult human scalp skin in organ culture, using a sensory skin reinnervation model (primary rat dorsal root ganglia neurons; serum-free, supplemented DMEM/F12) and focussing on immunohistologically detected, rat-specific sensory NFs (PGP95+/ratMBP+) that were in close contact with tryptase+ and/or c-Kit+ MCs and compared re-innervated with denervated skin over 5, 11 and 17 days of organ culture.. This showed that the number of both myelinated (MBP+) and non-myelinated rat nerve fibers increased steadily during culture, first around hair follicles and then in the vicinity of skin MCs and the epidermis (which was fully reinnervated by day 11). c-kit+ or tryptase+ MCs were preferentially contacted by myelinated fibers, and their number was significantly higher in reinnervated skin compared to denervated skin during all time points (MC degranulation was largely unaffected). With progressing culture time, the number of c-Kit/tryptase double-positive cells further increased, suggesting the maturation of resident, c-Kit+/tryptase- MC progenitors *in situ*. The effects on MC survival/proliferation, NF neuropeptide expression profile, and the underlying mechanism(s) of action are currently being investigated. These findings already suggest that, just as in mice *in vivo*, the sensory reinnervation of human skin promotes skin MC maturation (and possibly also survival). Thus, the sensory innervation status of human skin is likely to affect MC-dependent human skin physiology and pathology. The current assay provides a highly tractable system in which to interrogate and manipulate peripheral nerve-immune system interactions in healthy adult human skin.

383

Plasmacytoid dendritic cells is a key player during the initiation phase of alopecia areata in C3H/HeJ mouse

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Recently idea of the pathomechanism of alopecia areata (AA) has been regarded as a tissue-specific autoimmune disease. So far, interferon (IFN)-γ has been regarded as the most important key cytokine that may induce the collapse of hair follicle (HF) immune privilege, apotosis and the upregulation of CXCL10 in AA. In this study, we focus on the role of type I IFN, IFN-α, in the initiation of AA because viral infection, such as influenza virus, may induce AA onset by an overproduction of interferons (IFNs). In addition, AA universalis is started after the treatment with PEG-IFN-α-2b for HCV infection. In this study, we generated C3H/HeJ mice with AA by subcutaneous injection of activated lymphocytes obtained from lymphonodes with IL-2, IL-7, IL-15 and Dynabeads T-Activator CD3/CD28. Immunohistochemical staining revealed that IFN-α producing plasmacytoid dendritic cells (pDCs) densely distributed around HFs in not only lesion skin but also non-lesional skin obtained from C3H/HeJ mice with AA. Flowcytometric analysis showed the increased frequency of pDCs in skin-infiltrated cells of non-lesional skin from C3H/HeJ mice with AA. Real-time PCR also showed not only higher expression of IFN-α2 and IFN-α4 but also chemerin mRNA expression in C3H/HeJ mouse with AA. The potent chemotactic factor for pDCs "chemerin" may accumulate pDCs around hair loss lesions. *In vitro*, IFN- α inhibited the hair elongation of cultured murine vibrissae in Willium's E medium by Philpott's model. In addition, $H\text{-}2^K$ and CXCL10 expression were upregulated in cultured murine vibrissae by IFN-α. Imiquimod (IMQ) is the potent inducer of IFN-α *via* TLR7/9 on pDCs. IMQ applied C3H/HeJ mouse showed retardation of hair cycle stage with infiltration of $SiglecH^+$ pDCs with IFN-α expression. Furthermore, our idea was proved by AA induction with subcutaneous injection of pDCs isolated from lesional skin in C3H/HeJ with AA. In conclusion, pDCs may play a central role for the initiation of hair loss by over expression of IFN-α in initiating phase of AA.

384

Mechanism of the anti-acne actions of fatty acid amide hydrolase inhibitors on human sebocytes

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We have previously shown that locally produced endocannabinoids increase sebaceous lipid production in human sebocytes. Interestingly, however, administration of a non-psychotropic phytocannabinoid (cannabidiol [CBD]) as well as pharmacological inhibitors (URB597 and JP104) of an endocannabinoid degrading enzyme (fatty acid amide hydrolase [FAAH]), led to remarkable anti-acne effects (reduced lipogenesis, anti-proliferative and anti-inflammatory actions). Hence, we aimed at investigating the mechanism of the aforementioned unexpected beneficial effects of the FAAH-inhibitors. Lipid synthesis of human SZ95 sebocytes was investigated by Nile Red staining. Alterations in the gene expression were monitored by RT-qPCR and Western blot. We found that URB597 and JP104 down-regulated c-Myc (a key positive regulator of sebocyte proliferation) and nuclear receptor interacting protein-1 (NRIP1), down-regulation of which was proven to mediate lipostatic actions of CBD. Although anti-inflammatory actions of CBD were mediated by the adenosine A2a receptor-dependent up-regulation of tribbles homolog 3 (TRIB3) and inhibition of P65-NF-κB signaling, FAAH-inhibitors did not influence either TRIB3 expression or lipopolysaccharide induced NF-κB-activation. Instead, antagonists of peroxisome proliferator-activated receptor (PPAR)-α, -γ and –δ were equally able to prevent their anti-inflammatory action. Our ongoing FAAH RNAi studies intend to reveal if these actions are indeed directly coupled to the abrogation of FAAH activity or they are mediated by yet unknown off-targets. Collectively, these results strongly argue for that administration of FAAH-inhibitors, leading to down-regulation of c-Myc and NRIP1 and activation of PPARs, exert complex anti-acne effects on human sebocytes; therefore clinical studies are invited to exploit their potency as a promising, novel class of anti-acne agents.

385

Comparison of allogeneic bone marrow derived mesenchymal stem cell and adipose tissue derived mesenchymal stem cell therapy in porcine burn wound healing

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Burn injuries can have significant effects on the patient both aesthetically and physically. Rapid burn wound closure is well correlated with survival, improved aesthetic appearance, decreased adverse or hypertrophic scarring and reduced risk of infection. Mesenchymal stem cells (MSC) have been shown to be efficacious in the treatment of cutaneous wounds and thermal injuries and recently, we have shown that bone marrow derived MSC can accelerate wound closure in a porcine animal model of burn wound healing. Consensus on the optimal source of mesenchymal stem cells has yet to be found. Several studies have shown that adipose tissue harbours mesenchymal stem cells (ASC) which can accelerate wound healing. Adipose tissue provides a much more clinically attractive source of MSC due to its ease of isolation and availability. In this study we compared the efficacy of MSC and ASC in a large porcine model of burn wound healing. Both ASC and MSC were isolated from the same donor landrace pigs. These cells were than characterised by the expression of cell surface receptor markers associated with MSC and were capable of differentiation into the various osteo- chrondo- and adipo-lineages. The cells ($5x10^6$/wound) were then delivered topically in a fibrin hydrogel onto the induced thermal contact wounds (4.5 cm^2) on the animal's back. Each animal had 12 wounds, 4 wounds per group (ASC, MSC and Fibrin alone). Analysis of the wounds after 14 days demonstrated that application of either cell therapy accelerated wound closure compared to fibrin alone. Both cell therapies induced an increased angiogenic response and stimulated collagen production. We conclude that ASC are as efficacious as MSC in burn wound healing and adipose tissue provides a clinically relevant source of these stem cells.

386

A trial to clarify the effect of the filaggrin gene mutation to keratinocytes biology by using CRISPR/Cas9 system and human induced pluripotent stem cells

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Previously, we have already successfully generated transgene-free and mutation-free human iPSCs (hiPSCs) from human dermal fibroblasts by using the *piggyBac* transposon system. Moreover, we successfully differentiated these hiPSCs into epidermal keratinocytes (iKCs; induced keratinocytes). Incidentally, recent advances in the development of genome editing technologies based on programmable nucleases such as zinc finger nucleases (ZFNs), transcription activator–like effector nucleases (TALENs) and the clustered regularly interspaced short palindromic repeat (CRISPR)-associated nuclease Cas9 (CRISPR/Cas9) have substantially improved our ability to make precise changes in the genomes of human cells. With our established systems of obtaining iKCs from hiPSCs and new technology of programmable nucleases, especially CRISPR/Cas9 system, we tried to clarify the precise effects of filaggrin gene (*FLG*) mutations to keratinocytes biology. A guide RNA that targeted appropriate site of human *FLG* was designed by web-based tool and cloning into the backbone vector of CRIPSR/Cas9 (h*FLG*-CRISPR/Cas9). We transfected h*FLG*-CRISPR/Cas9 into hiPSCs and obtained the several clones of hiPSCs which possessed random mutations in *FLG*. Then, original hiPSC and *FLG*-mutated hiPSCs were differentiated into epidermal keratinocytes using our established protocols and we obtained the normal iKCs and *FLG*-mutated iKCs. Under this condition, we can compare the phenotypes of normal and *FLG*-mutaed iKCs of the same genetic background. Therefore, the results obtained from this system should be "true" meanings of *FLG* mutation to keratinocytes biology and should be important information for the understanding of AD pathogenesis.

387

Gradual Development of Blood and Lymphatic Endothelial Cells in Prenatal Human Skin

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Adult human skin is a highly vascularized tissue containing both blood vessels (BVs) and lymphatic vessels (LVs) that initially form during intra-uterine life to give rise to anatomically and phenotypically distinct networks. In this study we investigated the development of blood and lymphatic endothelial cells in prenatal human skin *in situ* using multi-color immunofluorescence and analyzed angiogenic molecules by protein arrays of lysates and cell culture supernatants. We found that between 8-10 weeks estimated gestational age (EGA), $CD144^+$ vessels predominantly express the venous endothelial marker PAL-E, whereas $CD144^+$PAL-E^- arteries only appear at the end of the first trimester. While lymphatic progenitor cells express CD31, CD144, Prox1 and temporary PAL-E, not all of them express podoplanin or Lyve-1 at 8 weeks EGA but acquire these markers with advancing gestational age. Already in second trimester human skin the phenotype of BVs and LVs is similar to the one in adult skin. The expression pattern of angiogenic molecules in prenatal skin did not show the expected bent to proangiogenic molecules, indicating at a complex regulation of angiogenesis during ontogeny. In summary, we found that the vascular network at the earliest developmental stages investigated is tilted towards a venous vessel phenotype and that the phenotypic repertoire of both BVs and LVs in second trimester human skin resembles the one in adult skin.

388

A metabolic cellular profile in vitiligo

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Previously, we showed that vitiligo melanocytes may be affected by a mitochondria-driven degenerative process leading to a pre-senescent phenotype and influencing the capability to cope with stressful stimuli. Mitochondria may be the source of the uncontrolled ROS production, giving rise to intracellular proteins modifications, which generate antigenic epitopes and consequently an autoimmune response. Now, in vitiligo melanocytes (VHM) (n=10 vs 8 normal (NHM) ones) we searched for index of mitochondrial impairment pointing on possible alterations of the energetic metabolism through a dissection of the steps of the glucose metabolism. VHM were characterized by lower ATP production (-40%) together with increased expression of the master mitochondrial regulator PGC1a (+15%) as well as of the permissive FAK phosphorylated (Y397), which ensure the generation of the active PGC1a/FAK complex at nuclear level to induce mitogenesis. Accordingly, we found increased total mitochondrial mass and a deregulated expression of some regulators of the mitophagic process. The upstream PGC1a activator, SIRT1, was also up-regulated. The mitochondrial and energetic involvements were further dissected by analysing the fission/fusion process, through the expression of MFN1/2, and the activity of the key glycolytic enzymes. The expression of the glycolytic enzymes hexokinase II, pyruvic dehydrogenase kinase 1, and pyruvic kinase M2, was also significantly higher in VHM. Exposure to oxidant agents (tBOOH) did not modify their expression in VHM whereas it produced a significant induction in NHM. Therefore VHM may produce sufficient energy in steady-state conditions but they were unable to cover further needs. Glutamine addition further increased ATP production in NHM but was ineffective in vitiligo ones. All these data indicated the existence of a metabolic mitochondrial inherited defect in vitiligo melanocytes that could be the biochemical basis for the disease.

389

Lrig1 and CD44v3 expression in human folliculosebaceous unit

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The expression of Lrig1 in human epidermis is described as clusters of Lrig1+ keratinocytes in the basal layer of the interfollicular epidermis (IFE). In contrast to human, the expression of Lrig1 in mouse is located in the isthmus connecting the infundibulum and the sebaceous glands (SG) of the folliculosebaceous unit (FSU). These Lrig1+ cells were shown to feed the FSU, and all the epidermis only upon injury. We have detected similar Lrig1+ clusters in the isthmus and SG of the human FSU, and studied the expression of epidermal growth factor receptor (EGFR) and CD44v3 in these clusters, since Lrig1 is an inhibitor of EGFR and hyaluronate (HA) receptor CD44v3 has been shown to modulate the EGFR signaling. The previously described clusters of Lrig1+ cells in the IFE were detected in the basal layer, and found to express less EGFR and CD44v3. In addition, we found Lrig1+ clusters in the isthmus and the SG. This suggests that two clusters of Lrig1+ keratinocytes are present in human epidermis, (i) one in the IFE as previously described, and (ii) a second follicular, similar to the murine follicular Lrig1 niche, which may have similar functions. In the mouse skin, in homeostatic conditions the IFE self-renew autonomously and the Lrig1 niche feeds the upper FSU only. The human epidermis may have evolved towards two distinct epidermal Lrig1 niches, one in the IFE and another one in the follicle. More scattered hair follicles in human skin may explain why human epidermis needs for an additional reservoir of Lrig1+ quiescent cells. In addition, the reduced expression of CD44v3 and EGFR in the Lrig1 niche may mean that the Lrig1+ cells reside in a specific extracellular matrix (ECM) or niche, as CD44 and EGFR activity were shown to affect the HA content of the ECM. This niche may be of interest in the understanding of the IFE and sebaceous gland homeostasis, and be involved in skin disorders associated with each compartment.

390

Crosstalk of the corticotropin-releasing hormone and substance P signaling pathways in cultured human dermal papilla cells

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Recently, several reports showed the relationship between stress hormones and neuroendocrine signaling. Corticotropin-releasing hormone (CRH) plays an important role in regulation of central and local stress responses along the hypothalamic-pituitary-adrenal (HPA) axis. Substance P (SP) is also an important mediator of an acute local and systemic stress responses through neurogenic inflammation. In hair organ culture system, CRH or SP inhibited hair growth and altered hair cycle via regulation of proliferation and apoptosis of outer root sheath cells. Although the crosstalk between CRH and SP signaling was reported in mast cells, there was no evidence about a link between CRH and SP in hair follicle cells so far. The objective of our study is to investigate the correlation between CRH and SP signals in cultured human dermal papilla cells (hDPCs) which play pivotal roles in controlling hair growth and hair cycle. Human hair follicles were cultured and exposed to varying concentrations of SP. SP significantly inhibited hair shaft elongation and induced early catagen transition. To understand underlying mechanism of SP, the effects of SP on cultured hDPCs were monitored. SP regulated the expression of the hair growth-related cytokines in hDPCs. Expression of CRH receptors (CRHRs) was increased by SP treatment in cultured hDPCs both in mRNA and protein levels. Likewise, treatment of CRH induced the expression of NK1, a SP receptor. Interestingly, hDPCs were more sensitive to SP when pretreated with CRH, showing lower levels of anagen-related signals. These results indicate that CRH and SP signals have a connection in cultured hDPCs, and suggest that their connection could induce mutual synergistic effect of other signal transmission in stress-induced hair loss.

391

Immortal murine dermal papilla cells introduced by TERT and Bmi1 genes maintain hair inductive activity

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Dermal papilla cells (DPCs) play an important role on the regeneration and development of hair follicles. Once DPCs are put on culture, hair inductive activity and proliferation of DPCs are rapidly lost due to cellular senescence induced by various biological stresses. In this study, we focused on two genes, telomere reverse transcriptase (TERT) and Bmi1. Surveillance mechanism of telomere length exists and cellular senescence is caused by telomere shortening. The length of telomere is kept maintaining by TERT. Furthermore, two proteins called P16 and P21 have an important role on cell arrest. Bmi1 is thought to avoid cell arrest by inactivating p16 because Retinoblastoma (RB) protein phosphorylated by cyclin dependent kinase (CDK) is kept dephosphorylating by activated P16 and P21 and cell cycle irreversibly stops. Therefore we introduced TERT and Bmi1 genes to murine vibrissal DPCs using a lentivirus vector and successfully produced immortal DPCs. To assess hair inductive activity of immortal DPCs, we performed hair reconstitution assay and demonstrated hair follicles induction by implanted immortal DPCs. Moreover, mRNA of immortal DPCs was compared with intact DP and cultured DPCs by microarray analysis.

392

Notch1 maintains stemness and stimulates keratinocyte proliferation during ageing of human keratinocytes in vitro

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The Notch signaling pathway regulates the development and homeostasis of a variety of tissues, including skin. Notch activities are complex and diverse depending on the cell context. In addition, Notch functions have been mostly investigated in genetically-modified mice. The aim of the present study is to evaluate the role of Notch1 in normal human keratinocytes in relation to ageing, with special regard to stem cells (KSC). We first demonstrated that Notch1 expression decreases during ageing in vivo. The activated form of Notch (N1ICD) was highly expressed in KSC and tends to decrease in transit amplifying (TA) cells to completely disappear in postmitotic cells in skin samples from young, adults and old subjects. Ca^{++} addition markedly downregulated N1ICD expression in keratinocytes. More specifically, Ca^{++} reduced the Notch1 activation in subconfluent keratinocytes from donors of all ages, while upregulating keratin10 (K10) and involucrin in the same samples. Gamma secretase inhibitor (DAPT), that blocks Notch activation, induced a dose-dependent increase in K10 and involucrin expression, while it caused a decreased expression of survivin. Moreover, DAPT reduced S phase of the cell cycle, while decreasing keratinocyte proliferation in a time- and dose-dependent manner both in adult and old subjects. In addition, DAPT decreased cell proliferation that was significantly more evident in young KSC than in old KSC. Finally, Notch1 silencing decreased survivin expression both in young- and old-derived KSC and TA cells, while N1ICD overexpression decreased involucrin and increased survivin levels. On the other hand, survivin overexpression increased Notch1 activation in KSC. In conclusion, Notch1 seems to contribute to the maintenance of stemness and the proliferation capacity in human keratinocyte during ageing.

393

Expression map of three distinct skin fibroblast populations isolated from human skin

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There are three distinct fibroblast populations found in adult skin dermis; papillary fibroblasts, reticular fibroblasts and dermal papilla fibroblasts. Recently, these were shown to arise from a common cellular progenitor. To isolate these populations from human skin, fibroblast position is taken into consideration. Papillary fibroblasts are located just beneath the epidermis, while reticular fibroblasts are located in the lower dermis, juxtaposed to the dermal white adipose tissue. Dermal papilla fibroblasts are located at the base of the hair follicle. To isolate these populations, we use a scalpel blade to cut the dermis very close to the epidermis, and separate the papillary fibroblasts from the reticular fibroblasts for expansion *in vitro*. Dermal papilla fibroblasts are isolated by microdissection of hair follicle end bulbs. These three fibroblasts populations are defined by their spatial location, and are under the influence of the surrounding macroenvironment. We wanted to know if these three populations would maintain their distinct identities *in vitro*, once surrounding influential factors were removed. In order to understand the divergence of fibroblasts and how the cells are spatially organised in the skin, it is vital that we have markers that can be used to identify the different subpopulations of dermal cells both *in vivo* and *in vitro*. We performed immunofluorescence with a panel of several antibodies, which we predicted would be differentially expressed in the different populations. We found that Vimentin and Collagen I are expressed in all three fibroblast populations. Comparatively, Podoplanin and Dipeptidyl-peptidase IV were expressed specifically in papillary fibroblasts but not reticular fibroblasts. Therefore, we were able to show that despite their similar morphological appearances, these three fibroblast populations do maintain discrete identities once removed from the skin and grown in vitro. We were able to establish an expression map that can serve as a platform for identifying and characterising these three different fibroblast populations *in vitro*, using human cells.

394

Dickkopf 1 from Dermal Papilla Cells may contribute to impair hair follicle stem cell differentiation in androgenetic alopecia

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Androgenetic alopecia (AGA) is the most common type of alopecia in men. Androgens action causes HF miniaturization and baldness through mechanisms which remain unclear. Hair follicle (HF) formation begins when signals from the mesenchyme-derived dermal papilla cells reach multipotent epidermal stem cells in the bulge region (HFSC). We previously reported that androgens abrogate dermal papilla-induced hair follicle differentiation via the inhibition of the canonical Wnt signalling pathway suggesting that androgens deregulate DPC-secreted factors involved in normal HF stem cell differentiation. On the other hand, Dickkopf 1 (DKK-1), a Wnt antagonist inducible by dihydrotestosterone (DHT) from balding papillae, promotes hair regression and causes apoptosis in follicular keratinocytes. The aim of this work was to determine if DKK-1 is involved in the differentiation of HFSC. DKK-1 mRNA expression induced by DHT was compared in androgen responsive dermal papilla cells (DPC) cultured as monolayer or as spheroids cell aggregates. In both culture conditions DHT induced approximately 40 times the expression of DKK-1 mRNA. Nevertheless its basal expression level was significantly lower in spheroid culture conditions. hDkk-1 treatment in DPC revealed a decrease in the cytoplasmic total b-catenin protein ratio indicative of canonical Wnt pathway inhibition. We used the media conditioned by DPC to induce HFSC hair-linage differentiation. Conditioned media obtained from DPC, cultured in presence of DKK1, lost its differentiation ability similar to what it was observed in DHT presence. Same results were obtained when conditioned media obtained from DPC were supplemented with DKK-1. These results suggest that DKK-1 may be one paracrine factor induced by DHT that contribute to abrogate hair linage differentiation of HFSC differentiation explaining in part the pathological effect of androgen in AGA.

395

The transcriptome of Lgr6+ epidermal stem cells in mouse skin

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Adult stem cells are a focus of research on developmental skin biology and skin carcinogenesis attributable to their life-long critical role in epidermal renewal. This study is aimed at a thorough characterization of stem cells carrying the Lgr6 receptor (cofactor of Frizzled in Wnt signalling) from the skin of hairless mice. As Lgr6 proved extremely difficult to detect by immunostaining, alternative (membrane) markers are needed. Epidermal cell suspensions were prepared from transgenic Lgr6-eGFP hairless mouse strains (Tg/Tg and Tg/wt). Basal cells (integrin α6+) were sorted by eGFP expression (Lgr6+ vs -). Quantitative RNA deep seq and gene set enrichment analysis was performed on α6+/Lgr6- and α6+/Lgr6+ cells. Quantitative analysis of the RNA seq data showed upregulation of stem cell related genes (e.g. Abcc5, Bmp2, Dll1, Ets1, Fgfr2, Fgfr3) in Lgr6+ cells. Moreover, the majority of the Wnt (related) genes (except for Wnt11) appeared to be up-regulated in Lgr6+ stem cells, while many non-coding RNAs were down-regulated (e.g., very prominently Snord15a and Snord15b in the Rps3 locus). In addition, we identified a set of up-regulated genes (e.g. Crispld2, Skint7, Slc2a12) in Lgr6+ cells that might be potential new markers for their identification. After successful isolation through FACS, RNA seq showed that Lgr6+ stem cells display an increased expression of RNAs encoding proteins involved in Wnt signalling — which is considered a hallmark of stem cells— and a down-regulation of a collection of non-coding RNAs. Thus, we have established a clear RNA profile of Lgr6+ cells which may serve further characterization and identification of these cells, also in human skin.

396

Endoreplication in the Human Hair Follicle?

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Cell cycle dynamics within the human hair follicle (HF) are still unclear. Endoreplication is a cell cycle phenomenon whereby sustained cycles of DNA replication occur in the absence of cell division, resulting in polyploidy. This alternative cell cycle may prevent aberrant cell proliferation, and is typical of differentiating cells that need to produce large amounts of RNA and protein. While strong evidence indicates that terminally differentiating human epidermal keratinocytes (KCs) undergo endoreplication, it is unknown whether this also occurs in human HFs. Since human scalp HF KCs continuously keratinize during the (exceptionally long) anagen growth phase, we asked whether they undergo endoreplication. Here we present evidence in line with the concept that they do. There is a typical endoreplicative nuclear size increase in suprabasal outer root sheath (ORS) KCs. EdU labelling highlights that DNA synthesis is abundant not only in proliferating matrix and basal ORS KCs, but also in suprabasal ORS KCs. Mitotic markers (phospho S10 H3 and phospho S780 Rb) were expressed in cells of both the human hair matrix and ORS, while the G2/M markers, Cyclin A and B, were primarily expressed within the matrix, suggesting that these cells are undergoing nuclear division. However, preliminary data suggest that, just above Auber's line, matrix KCs maintain their S phase and continue to express Cyclin E and Ki67, while they downregulate G2/M cyclins. Furthermore, in situ analyses revealed chromosomal amplifications. Taken together, this supports that some differentiating human HF KCs could engage in endoreplication. Moreover, contrary to a long prevailing view, human ORS KC differentiation may coincide with continued cell cycling, as opposed to immediate cell cycle arrest.

397

First indications that growth hormone plays a role in human hair follicle biology

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Growth hormone (GH) and its receptor (GHR) promote cell growth, proliferation, differentiation and stem cell activation either directly or via the induction of IGF-1. While some clinical case reports suggest that the hair follicles (HFs) may be GH-responsive, the function of GH in human hair biology remains undefined. In order to better characterize it, we initially performed GHR immunohistochemistry in human scalp skin and found GHR to be expressed in the epidermis and in the epithelium of an anagen IV HFs as well as in the sebaceous gland. In organ-cultured human scalp HFs, qRT-PCR analysis revealed a significant decrease in the expression of GHR in catagen HFs compared to anagen VI. The expression of GHR was inversely correlated with that of somatostatin (GH-inhibiting hormone), whose expression increased in catagen HFs compared to anagen. In addition, organ-cultured human scalp HFs were stimulated with recombinant hGH (rhGH, 50-100 ng/ml) or recombinant GH-binding protein (rGHBP). GHBP which is derived from the cleavage of the extracellular domain of GHR, can exert both GH-antagonistic and agonistic effects. After stimulation of HFs, interestingly, hGH and GHBP both promoted hair follicle elongation. Hair cycle staging showed that both GH and GHBP-treatment appeared to retard catagen development. qRT-PCR analysis showed that hGH (100 ng/ml) and GHBP (100 ng/ml) induced an increase in levels of JAK2, which is the downstream target after GH ligand binds to GHR. Interestingly hGH also increased the levels of IGFBP3, which has been shown to be lower in patients with vertex balding. In addition levels of IGF-1 were relatively unchanged after stimulation with both hGH and GHBP. This pilot study suggests that GH-induced signaling is a novel endocrine regulator of human hair growth *ex vivo* and may be targeted therapeutically.

398

Expression patterns of clock and clock controlled gene mRNAs in psoriatic skin lesions

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The 24 hour (circadian) clock harmonizes cellular functions to the constantly changing environmental cycles, and activates a large propotion of our genes in a daily rhythm. As skin is a barrier organ, it is continuously exposed to diurnal changes in environmental conditions. Epidemiological studies have shown that the disruption of the circadian system (e.g. shift work) leads to an increased risk of certain skin conditions (psoriasis, skin tumors). The aim of this study was to investigate the daily expression patterns of circadian clock genes (*per1, per2, clock,* and *cry1*) and clock controlled genes (Rev-erbalpha, Dbp, c-myc) in human skin biopsies obtained from psoriatic lesion and from non-symptomatic psoriatic skin. 3 mm punch biopsies were obtained from 8 volunteers suffering from psoriasis. The samples were collected from non-involved (and not treated) and involved skin lesion at 3 time points during the day. Expression of clock genes and clock controlled genes were determined by qRT-PCR. The mRNA expression of bmal-1 and cry1 differed in psoriatic skin lesion compared to non-lesional skin at the different timepoints. Daily expression pattern of the clock-, and clock controlled genes were analysed and our preliminary results will be presented in the context of recent knowledge on the role of clock genes in skin physiology To our knowledge this is the first study to investigate the rhythmic expression of clock genes in psoriatic lesions throughout 24 hours. Our results and future studies may shed light on the application of chronotherapy (certain time of the day, when efficacy is highest and side-effects are lowest) in dermatology. Identification of time frames of the day with optimal or suboptimal states of skin physiology may have impact on the timing of dermatological treatments (e.g. when to administer UVR, optimizing steroid application in a daytime manner).

399

Do γδ T cells contribute to human hair biology and pathology?

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γδ T cells are key protagonists of the murine skin immune system regulates hair follicle (HF) cycling and neogenesis. However, their characteristics and functions in human HF biology and pathology remain completely unknown. As a first step towards elucidating their role, the distribution and number of **γδ** T cells was characterized in human scalp skin (n=15). As expected, **γδ**TCR+ cells were detected in the epidermis and in the epithelium of anagen and telogen HFs. Similar to murine HFs, their intrafollicular expression was strikingly restricted to the distal HF epithelium above the isthmus in anagen and telogen HFs. Interestingly, almost no **γδ**TCR+ cells could be visualized in human catagen HFs. Peri- and intrafollicular **γδ** T cells in healthy human skin were **Vδ1**+. Autologous, skin-derived human **γδ** T cells induced HF cytotoxicity (LDH assay) and up-regulated ectopic MHC class I expression, if co-cultured with (stressed?) HFs. In patients with alopecia areata (AA) (n=5), **γδ**TCR+ cells not only densely populated the perifollicular inflammatory cell infiltrate of lesional HFs (AA vs. healthy skin, 5.0±0.8 vs. 0.2±0.0; mean ± SEM), but also prominently infiltrated into the hair bulb (7.5±3.8 vs. 0.0±0.0). Interestingly, the hair bulb-infiltrating **γδ** T cells in lesional AA HFs also expressed **Vδ1**TCR. While **Vδ2** T cells have been implicated in the pathogenesis of human autoimmune disease, an involvement of **Vδ1** T cells is a novel finding, suggesting that **Vδ1** T cells may play a hitherto unknown role in AA pathobiology. We expect to report the results of ongoing studies whether **γδ** T cell secretory activities, surface marker expression profile, and cell-cell interactions change between AA HFs and healthy controls. For these analyses, we also developed a new culture medium that prolongs viability of both, human **γδ**TCR+ cells *in situ* and of organ-cultured human HFs. Besides perifollicular mast cells and macrophages, **γδ**TCR+ cells may be another important innate immunocyte population in human HF biology and pathology.

400

The sebaceous gland shows differential responses to spontaneous and induced cycling

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Historically the sebaceous gland (SG) was considered to be a dispensable compartment of the pilosebaceous unit, having little impact on hair follicle (HF) homeostasis – an assumption which has recently been challenged. In order to further our understanding of the relationship between HF and SG, morphological and molecular parameters within the SG, during both a depilation-induced and spontaneous murine HF cycle, were analysed. During induced cycling, SG morphology changed drastically, with significant increases in SG area, sebocyte area and the total sebocyte number per gland at mid-anagen ($P<0.001$). Significant differences in the levels of lipid staining intensity were also observed. There was a significant peak in proliferation ($P<0.05$) during mid-anagen, with no significant changes in levels thereafter. By comparison, during spontaneous HF cycling the morphological changes observed within the SG were much more subtle. SG area fluctuated only slightly, with a significant increase in size observed between late catagen and telogen ($P<0.01$). Sebocyte area decreased only between mid catagen to late catagen ($P<0.001$) with no significant differences in numbers of sebocytes observed. Despite the small changes in SG morphology, fluctuations in lipid staining intensity and expression of SG specific markers were evident. The expression of Perilipin 2 significantly increased from mid anagen to late anagen ($P<0.001$) and from early catagen to mid catagen ($P<0.001$). These results suggest that during spontaneous HF cycling, the SG maintains a set morphology. After HF trauma, such as hair shaft plucking, the SG responds by increasing overall size, demonstrating a synergistic, inter-dependent relationship between the HF and SG.

401

Transcriptome analysis of molecular signaling pathways in androgenetic alopecia

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The male androgenetic alopecia (AAGM) is hereditary in more than 80% of the cases. The hair loss is progressive and starts in the frontal area and the vertex, with a prevalence increasing with age. We aimed to identify molecular biomarkers associated with premature AAGM in order to detect targets for future treatments. The study was monocentric and included 18-35 years old Caucasian volunteers in 2 groups: *"A"*: 15 hairless/bold men with premature AAGM (stage V-VII in Norwood classification), *"C"*: 15 control healthy men (<2% white hairs, stage I- II). Scalp biopsies (2mmØ) were carried out on the vertex at the alopecia area edges for "A" and similar emplacement for "C". Gene expression analysis was performed using Agilent Whole Human Genome Oligo Microarrays (onecolor 8x60K v2). Characterization of the gene expression profile differences between the 2 groups was assessed by pairwise comparisons between A & C using a discriminatory genes analysis and a functional annotation analysis was used to provide an overview of the biological processes and pathways. The results evidenced 325 up-regulated gene sequences in "A" group vs "C", with only 178 genes annotated in the functional analysis, leading to a final number of 149 conserved genes. Conversely, 184 genes were significantly down-regulated in A *vs* C. Several gene pathways - including those involved in stem cell fates and development, such as the Wnt-βcatenin pathway, vitamin D metabolism, keratins, POMC and TGFβ-associated pathways - were significantly decreased in A *vs* C. In contrast, mast cell granule-derived enzymes, inflammatory mediators and immunoglobulin-associated immune effectors were significantly up-regulated. The validation of candidate genes was obtained by alternative methods: qRT- and fluidic-PCR and some marker immunostaining on scalp biopsie frozen sections. Altogether, this study provides evidence of alopecia molecular events that might be used as target for new efficient treatments.

402

Impact of a stimulating shampoo treatment on the penetration of Minoxidil through hair follicles

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Androgenetic alopecia (AGA) in men presents clinically with reduced hair density and hair width due to shortening of the hair cycle in genetically predisposed scalp areas The effectiveness of hair growth-promoting agents, like Minoxidil, in AGA has been demonstrated in numerous randomized controlled trials. Ours laboratories developed a stimulating cream shampoo that prepares the scalp to reactivate its vital functions and those of the hair. The stimulating shampoo can be used before application of Minoxidil solution and help the penetration of Minoxidil following topical application. The aim of this study was to investigate the difference of rate and extent of the *in-vitro* penetration through hair follicles of Minoxidil following topical application with and without pre-treatment with the stimulating shampoo. The skin penetration study was realized at finite conditions (10µl/cm^2), on human scalp at two different times (10 and 30 minutes after Minoxidil application). The human scalp was mounted on dynamic cells using radiolabelled compounds (^{3}H-Minoxidil). ^{3}H-Minoxidil was quantified in the different compartments of the skin (surface, *stratum corneum*, hair follicles, remaining skin and receptor fluid) by scintillation counting. The mass balance was in accordance with guidelines (100 ± 15%) and permits to validate the study. After 10 and 30 min, higher quantities of Minoxidil were recovered into the hair follicles and scalp after pre-treatment with the stimulating shampoo. Indeed, the percentage of Minoxidil recovered into the hair follicles was 0.16% and 0.06% of the applied dose, with and without shampoo pretreatment, respectively. In the scalp, the percentage of Minoxidil reached 2.68% and 0.68% of the applied dose, with and without shampoo, respectively. The data obtained permits to conclude on the enhancing effect of the stimulating shampoo on Minoxidil penetration and help to target the hair follicles.

403

Glyceryl laurate and Acetyl tetrapeptide-3 combination as a new potential treatment of androgenetic alopecia

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Androgenetic alopecia (AGA), the most common form of alopecia in men, is an androgen-related condition in genetically predisposed individuals. The increased concentration of dihydrotestosterone (DHT) in balding scalps is responsible for the cross-talk between the dermal papillae and the hair follicle (HF) epithelial cells which leads to miniaturization of HFs and delays anagen onset. The aim of this study was to evaluate the effect of two active ingredients, namely Glyceryl laurate (GL, Monolaurine) and Acetyl tetrapeptide-3 (ATP-3, Peptidoxyl-4), and their association on the main factors modulating hair growth and being deregulated in AGA. Human dermal papilla cells (DPCs) were incubated with GL to assess 5-alpha-reductase gene expression (qRT-PCR analysis) and activity (TLC analysis of [^{14}C]-testosterone metabolization). In other experiments, DPCs were incubated with ATP-3 and the expression level of Vascular Endothelial Growth Factor (VEGF) was measured in cell culture supernatants (ELISA). Finally, microdissected human scalp HFs were incubated with GL, ATP-3 and their association for gene expression analysis (qRT-PCR). GL inhibited both 5-alpha-reductase gene expression and activity in DPCs. We found that ATP-3 enhanced VEGF production in DPCs up to 136%. qRT-PCR analysis of microdissected human scalp HFs revealed that the association of GL and ATP-3 could modulate synergistically frizzled class receptor 1 (FZD1) and secreted frizzled-related protein 1 (SFRP1) mRNA, two important modulators of canonical Wnt signalling in HFs. In conclusion, GL inhibits DHT production in DPCs which is increased in AGA and responsible for numerous molecular cross-talks. The inhibition of VEGF expression in DPCs and the down-regulation of canonical Wnt pathway in HFs are among the main disruptions caused by DHT and responsible for the delay of anagen onset and the decrease of its duration. The association of GL and ATP-3 is a good candidate for AGA treatment as ATP-3 stimulates VEGF production in DPCs and as the synergy of GL and ATP-3 could help to restore canonical Wnt pathway in HFs.

404

Slimming and restructuring effect of a Maca leaves extract: from *in vitro* efficacy to clinical proof

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The search for a slim and toned body is a universal request. Biologically, at the skin level, both dermis, hypodermis and microcirculation are involved. We developed and patented a polyphenols-rich extract from Maca leaves and demonstrated its slimming and restructuring potential *in vitro* and *in vivo*. On endothelial cells, the extract stimulated VE-cadherin staining, inhibited ICAM-1, VCAM-1 and E-Selectin expression (qPCR) and decreased adhesion of macrophages U937showing an ability to strengthen blood vessels and maintain vascular tone. The extract reinforce and protect the dermal matrix ; as shown by the increase of collagen I, elastin and decorin by fibroblasts (qPCR, ELISA) and inhibition of elastase release by neutrophils; resulting in a tensing effect measured by the Glasbox® system. Moreover, the extract stimulated lipolysis and inhibited lipogenesis in adipocytes and decreased the fibrotic condition of the adipose tissue as shown by fibronectin decrease (western blot) in inflammatory pre-adipocytes. *In vivo* effects of the extract on the body morphology and skin properties were assessed in a double blind study performed on 33 women volunteers. After 28 days, twice daily application of the extract in formulation showed a significant improvement of the behavior of the skin compared to the placebo product, as measured by Cutometer on thighs: the skin was more elastic and more tonic. Results at day 56 showed a significant decrease of the belly and thighs volume traducing a slimming effect, measured by the fringe projection technique. Significant difference compared to the placebo product effect was observed. By its reinforcing effect on the dermis and blood vessels associated to its action on adipocytes, the extract could increase skin firmness, maintain vascular tone and ease fat elimination. *In vitro* observations were confirmed by clinical results revealing the slimming and restructuring effects of Maca leaves extract.

405

Adenosine triphosphate, a new candidate for reactional hair loss treatment

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Reactional hair loss occurs few months following an acute psychological stress or important hormonal changes. This increased diffuse hair shedding is caused by the synchronized entry into telogen phase of numerous anagen hair follicles (HFs). Adenosine triphosphate (ATP) is a universal endogenous molecule known to constitute an essential energy source for cells. The aim of this study was to evaluate the effect of ATP on anagen onset key factors expression in HFs. Microdissected human scalp HFs were incubated with ATP for 6 or 48 hours (gene expression analysis using qRT-PCR). Human dermal papilla cells (DPCs) were incubated with ATP for 24 hours and the expression levels of Keratinocyte Growth Factor (KGF) and Bone Morphogenetic Protein 6 (BMP6) were measured in cell culture supernatants using ELISA analysis. qRT-PCR analysis revealed that ATP increased KGF and BMP6 mRNA levels in HFs up to respectively 251% and 373% compared to control HFs. KGF and BMP6 productions by cultured DPCs were also increased by ATP with a mean stimulation of respectively 37% and 44%. In conclusion, ATP increased KGF and BMP6 expression levels in HFs. KGF being a key growth factor for HFs development during anagen phase and BMP6 being important for HF induction by DPCs, the results of this study indicate that ATP is a good candidate for reactional hair loss treatment.

406

Growth of females scalp hair using LED source and vibration massage in female androgenetic alopecia

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Light emitting diode (655nm) has demonstrated a huge success in male apttern baldness, scalp massage after LED exposure was shown to prove slightly better responce than LED alone. Laser helmets have shown great practical and clinical responce in this process. Twenty females were chosen in that study ranging from 33-56 years old. Ludwig–Savin Baldness Scale I–II was used. they were (I-3, I-4, II-1) baldness patterns. they were divided in two groups, each group with ten ladies. group one was treated by LED only, Group two recieved LED with alternating vibration massage on the same session. they were subjected to LED 3 times weekly for 18-20 weeks. The results were evaluated by trichoscopy and it showed slightly difference in group two in the post treatment hair counts and density. Rate of hair growth was also slighty faster. Vibration massage improves the effect of LED 655 nm on the scalp concerning hair counts and density in women with androgenetic alopecia.